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Regulation of developmental transitions

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Plants undergo a series of profound developmental changes throughout their lifetimes in response to both external environmental factors and internal intrinsic ones. When these changes are abrupt and dramatic, the process is referred to as phase change. Recently, several genes have been discovered that play a role in these developmental transitions. Their sequence and expression patterns shed new light on the mechanisms of phase change, and provide a link between the external and internal factors that control them. Examples of these transitions include changes from juvenile to adult leaf formation, vegetative to inflorescence meristem development, and inflorescence to floral meristem initiation.

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Current Opinion in Plant Biology 2005, **8**:67–70

This review comes from a themed issue on
Growth and development
Edited by Liam Dolan and Michael Freeling

Available online 19th November 2004

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2004.11.002

Abbreviations

ago1	*argonaute1*
bd1	*branched silkless1*
BELL	**BEL1-like**
CO	*CONSTANS*
FLC	*FLOWERING LOCUS C*
fzp	*frizzy panicle*
id1	*indeterminate1*
miRNA	microRNA
pla1	*plastochron1*
PNF	POUNDFOOLISH
PNY	PENNYWISE
SAM	shoot apical meristem
VIN3	*VERNALIZATION INSENSITIVE3*

Introduction

Plant form changes over time in response to a variety of different factors. Such changes can be subtle and occur gradually or can be dramatic and occur suddenly. The latter situation is commonly referred to as phase change, and several groups have taken advantage of two model plant systems, maize and *Arabidopsis*, to study the genetics and molecular biology of this process. One central question regarding phase change is where the locus of change initiates within the plant. Because plant form is dependent on the activity of meristems, it is believed that phase change must involve a meristem-dependent component. Then again, developmental transitions are intricately tied to environmental factors that might influence the meristem indirectly. Several newly identified genes that are involved in these pathways reveal how the balance of meristem and non-meristem factors brings about the remarkable morphological transitions that characterize phase change.

Juvenile-to-adult transition in leaves

Several groups have described the histological and morphological differences between juvenile and adult leaves in *Arabidopsis* [1]. For example, early leaves are smaller and more rounded than leaves formed later in development. In addition, adult leaves have serrations and abaxial trichomes, whereas juvenile leaves do not. These phenotypic differences have been the basis for several genetic screens [2,3]. Similar mutant screens have also successfully identified phase-specific genes in monocots such as maize. For example, the *glossy15* gene of maize represses adult cell characteristics in the juvenile leaves of maize where the gene is expressed [4]. Interestingly, the genes thought to function in juvenile-to-adult leaf transitions in *Arabidopsis*, for example, the *SQUINT* gene that encodes a cyclophilin 40 chaperone protein, are broadly expressed in both the juvenile and the adult phases [5]. This fact might underline the differences in leaf differentiation between maize and *Arabidopsis*, or indicate that additional levels of regulation control this process in dicots.

The *hasty* mutant was identified in a screen for *Arabidopsis* mutants that cause the precocious production of abaxial trichomes on early leaves [2]. *HASTY* was cloned and shown to encode a widely expressed ortholog of the *exportin 5* gene of yeast [6•]. Exportin proteins export a variety of proteins, including both phosphorylated forms of several transcription factors [7] and double-stranded RNA-binding proteins [8]. Given that expression of the *HASTY* gene is not specific to the juvenile or the adult phases of development, a possible mode of function for HASTY in phase change might be indirect (i.e. the cargo that HASTY transports, rather than HASTY itself, could be involved in phase change). Recently, it was demonstrated that microRNA (miRNA) precursors are efficiently transported by Exportin 5 to the cytoplasm, where they are processed to 22-nucleotide miRNAs [9]. In *Arabidopsis*, such miRNAs might function in lateral organ polarity; for example, miRNA165/166 represses the *PHABULOSA* (*PHAB*) gene [10] that promotes adaxial

leaf identity. Consequently, it is possible that organ polarity genes are derepressed in *hasty* mutants because of faulty miRNA transport, leading to adaxialization of the leaf. Support for this hypothesis comes from the study of new alleles of the miRNA biogenesis mutant *argonaute1* (*ago1*). These *ago1* mutants have abaxial trichomes on early leaves as a result of ectopic expression of the *PHAB* transcript [11]. Thus, the change from juvenile to adult leaf characteristics is perhaps controlled by the spatial and temporal regulation of leaf polarity factors.

Another *Arabidopsis* phase-change mutant is *zippy*, which not only has adult leaf traits on leaves one and two but also shows pleiotropic defects in flower and carpel development. Double-mutant analysis places *ZIPPY* in the same pathway as *HASTY*, but in a parallel pathway to *SQUINT*. *ZIPPY* encodes a widely expressed member of the *AGO* gene family and, similar to *SQUINT* and *HASTY*, shows no juvenile phase-specific expression [12•]. In light of the facts that *AGO* genes function in a variety of miRNA processes and that HASTY might transport miRNAs, it is tempting to speculate that ZIPPY might also be involved in miRNA biogenesis. As in the case of *SQUINT* and *HASTY*, it seems likely that the genes controlled by *ZIPPY*, rather than *ZIPPY* itself, are the targets of phase change.

Vegetative-to-reproductive transition

The most dramatic example of A developmental transition in plants is the change from vegetative to reproductive development. The environmental factors that regulate flowering converge at the shoot apical meristem (SAM), and this convergence ultimately brings about the floral transition. Many single mutants have been described that alter the timing of flowering, but all of these mutants flower eventually. Recently, two new players in this transition were found, namely called PENNYWISE (PNY) and POUNDFOOLISH (PNF). *KNOTTED1-LIKE HOMEOBOX* (*KNOX*) genes are known to function within the meristem to maintain indeterminate cell identities [13,14]. KNOX proteins interact biochemically with other homeodomain proteins belonging to the BEL1-like (BELL) class [15–17]. When two members of the BELL family, PNY and PNF, were knocked out, a novel non-flowering mutant phenotype was revealed [18•]. The *pny pnf* double mutant expresses floral transition markers such as *SUPPRESSOR of OVEREXPRESSION OF CONSTANS* (*SOC1*) and *FRUITFUL*, indicating that floral inductive signals are received by the SAM and yet flowers are never made, even months after germination [18•]. The SAM of the *pny pnf* double mutant shows several defects in morphology, demonstrating that these *BELL* genes are necessary for the completion of the morphological changes in the SAM that allow it to respond to floral signals.

Recent work has led to a better understanding of how environmental factors such as photoperiod, which is perceived in the leaf, and cold temperature, which is perceived at the meristem, regulate flowering time [19,20]. *CONSTANS* (*CO*) is a key player in the regulation of flowering by photoperiod; wildtype *Arabidopsis* plants flower sooner in long days than in short days, whereas *co* mutants flower late in both short and long days [21]. Daylength is perceived in the leaves [22], yet how that signal moves to the meristem is a mystery. George Coupland's group [23••] recently showed that CO functions non-autonomously and that its expression in the phloem, but not in the meristem, is sufficient to directly activate the target gene, *FLORAL TIMING* (*FT*). These findings place CO in a good position to regulate a systemic flowering signal.

CO mRNA levels are regulated by the circadian clock, peaking in the evening. In long days, the peak is biphasic, with one peak occurring while there is still daylight [24]. This peak of *CO* expression requires the photoreceptor FKF1, an F-box flavin-binding protein [25•] that is probably a blue-light receptor. In addition, CO protein stability is antagonistically regulated by photoreceptors, being stabilized by blue and far-red light and destabilized in the dark and in red light [26•]. Thus, the coincidence of circadian-controlled mRNA peaks and protein stability ensures high levels of CO that activate the transcription of floral pathway integrators.

In *Arabidopsis*, cold regulates flowering through the floral repressor, *FLOWERING LOCUS C* (*FLC*; for review see [19]). Plants that have dominant alleles of *FLC*, a MADS-box gene, do not flower unless they have undergone a long and sustained cold period, known as a vernalization period. *FLC* RNA levels decrease during this cold period and permit flowering by no longer repressing genes such as *SOC* and *FT*. Recent analysis of *VERNALIZATION INSENSITIVE3* (*VIN3*) shows that this gene is a key player in the vernalization process. It encodes a PHD-finger protein whose expression is induced by a long cold period. As *VIN3* RNA levels increase, *FLC* levels decrease, allowing flowering. *VIN3* is expressed specifically in the meristem in the same pattern as *FLC*, and the VIN3 protein interacts directly with the *FLC* locus and represses its expression [27•]. VRN2 and VRN1, are also present in this pathway and are required to maintain the inactive state of *FLC* by histone methylation [28]. *FLC* is also negatively regulated by other genes, including *FVE*, which is a retinoblastoma-associated protein [29,30].

One of the few genes known to be responsible for the floral transition in maize is *indeterminate1* (*id1*) [31]. This gene encodes a unique zinc-finger protein that binds to an 11-base-pair, T-rich consensus sequence [32]. Like other late-flowering mutants, *id1* mutants produce many more leaves than the wildtype. When an inflorescence is finally made, however, vegetative seedlings are produced

amongst the floral structures, demonstrating that *id1* is necessary not only for initiating the floral transition but also for maintaining it. Surprisingly, *id1* expression was not observed within the SAM but within the young leaves surrounding it, indicating that ID1 functions non-cell autonomously with respect to the SAM [31]. *id1* and *CO* are clear examples of phase change genes that act at a distance from the meristem to transduce environmental signals to cause a developmental transition.

A similar mutant, called *plastochron1* (*pla1*), has been described in rice [33]. *pla1* was identified on the basis of its rapid initiation of vegetative leaves, although its time to flowering is unaffected. Like those of *id1*, *pla1* mutant panicle primordia display reversion to vegetative shoots. Hence, in addition to controlling the rate of leaf initiation, the *PLA1* gene also functions to repress vegetative development within the reproductive phase. The *PLA1* gene encodes a member of the cytochrome P450 gene family [34••]. Like *id1*, *PLA1* is not expressed within the meristem, and instead is found at the abaxial side of leaf primordia and bract leaves, and within the stem. It will be interesting to determine how the unique expression pattern of *PLA1* is able to coordinate the timing of leaf initiation within the SAM.

Inflorescence-to-floral transitions

In *Arabidopsis*, several well-described genes, such as *LEAFY*, are necessary for the inflorescence meristem to switch to the production of floral meristems [35]. In maize, the *LEAFY* gene is duplicated, and when both copies are mutated, defects in floral organ identity and determinacy are seen [36•] that are similar to those seen in dicots. These results suggest that the *LEAFY* genes have maintained a conserved role in floral development in monocots and dicots.

The *branched silkless1* (*bd1*) mutant of maize is also required for the transition from the inflorescence meristem to the floral meristem [37]. In the female inflorescence of *bd1* mutants, the spikelet meristem that normally initiates a pair of floral meristems becomes highly branched and behaves more like a branch meristem from the tassel. The *bd1* gene product belongs to the ERF class of transcription factors, and is expressed at the base of the spikelet meristem in the axil of the glume [38]. Surprisingly, *bd1* is not expressed within the spikelet meristem, although the identity of the meristem is altered in the mutant. The *frizzy panicle (fzp)* mutant of rice is phenotypically similar to *bd1* mutants, and also displays a conversion of spikelet meristem to indeterminate branch [39]. The *fzp* gene is the rice ortholog of *bd1* [40] and has an expression pattern that is similar to that of *bd1*. Expression of *fzp*/*bd1* in the axil of the glume may be required to repress the formation of axillary meristems, which are derepressed in the *bd1* and *fzp* mutants and take on branch-like qualities.

Conclusions

Plants successfully integrate several environmental signals to undergo developmental transitions. Although these transitions must involve the activity of the meristem at some point, the causative signals are most likely to come from outside the meristem. The fact that several genes that are necessary for these transitions, such as *CO*, *id1*, *PLA1* and *bd1*, are not expressed within the meristems that they control, provides some evidence for this hypothesis. The next challenge is to discover which external factors activate these genes, and how subsequent signaling to the meristem occurs to effect phase change.

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